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An External Quality Control Survey (EQCS) for Serum Cortisol

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Summary: The external quality control survey (EQCS) was based on those already published from this department (Marschner, I. et al. (1976) J. Clin. Chem. Clin. Biochem. 14, 345–351; Marschner, I. et al. (1974) Horm. Metab. Res. 6, 293–296; Horn, K. et al. (1976) J. Clin. Chem. Clin. Biochem. 14, 353–360). Each participant received 20 sera sent in dry-ice by express post or airmail, together with a detailed questionnaire requesting assay details and raw data from his own standard curve as well as from the 20 samples.

The data returned was transferred to punched cards and processed by computer (Siemens 404/3) using a modular system of programmes and sub-routines.

The serum samples contained a hidden standard curve, samples for assay internal control and sera from suppression and stimulation tests. Possible crossreacting substances were added to 2 sera. Each participant received a detailed computer print-out with an explanatory letter and set of histograms for each serum sample with which he could check his performance against other participants, in particular those using the same kit as himself. 87 laboratories received the 20 samples for cortisol assay. 64 laboratories returned data on the questionnaire sent, and of these, 49 sets of data could be fully worked out, the other 15 were only partly interpretable. Apart from laboratory-own methods, results from 9 different commercial kits were received. 21 participants had an intra-assay coefficient of variation under 5 %, 16 participants between 5 and 10 %. The minimum coefficient of variation for the 3 sera measured was 0 % and the maximum 86 %. Coincidence of laboratory-own and hidden standard curves was only seen in 13 cases.

Externer Ringversuch für Cortisol im Serum

Zusammenfassung: Es wurde ein Ringversuch nach früherem Modell (Marschner, I. et al. (1976) J. Clin. Chem. Clin. Biochem. 14, 345–351; Marschner, I. et al. (1974) Horm. Metab. Res. 6, 293–296; Horn, K. et al. (1976) J. Clin. Chem. Clin. Biochem. 14, 353–360) durchgeführt.

20 Seren wurden in Trockeneis verpackt und per Express an die Teilnehmer versandt. Jeder Teilnehmer erhielt einen Fragebogen, in dem alle Assay-Parameter sowie die Impulsraten der laboreigenen Standardkurve und die der 20 gemessenen Seren verlangt wurden. Die Ergebnisse wurden auf Lochkarten gestanzt, in einen Siemens 404/3 Computer eingelesen und ausgewertet. Die Auswertung erforderte etwa 30–35 Programme, die auf modularer Basis zusammengestellt wurden. Die 20 Seren setzten sich zusammen aus einer versteckten Standardkurve, Proben mit kreuzreagierenden Substanzen, Seren von Stimulations- bzw. Suppressionstesten und anderen Poolseren.

Jeder Teilnehmer erhielt einen Computerausdruck, einen ausführlichen Begleitbrief und einen Satz Histogramme, anhand derer er seine Leistung mit den anderen Teilnehmern vergleichen konnte.

Die 20 Serumproben für die Cortisolbestimmung wurden an 87 Laboratorien versandt. 64 Laboratorien sandten Ergebnisse zurück, von denen 49 voll auswertbar und 15 nur teilweise auswertbar waren. Neben laboreigenen Methoden wurden Ergebnisse von 9 kommerziellen Testbesteckten eingesandt. 21 Teilnehmer hatten einen Variationskoeffizienten (VK) unter 5 % und weitere 16 Teilnehmer einen VK zwischen 5 und 10 %.

Der geringste VK betrug 0 % und der höchste VK 86 %.

Eine Übereinstimmung der laboreigenen und der versteckten Standardkurve wurde nur in 13 Fällen erreicht.

Introduction

The need for external and internal quality control in hormone assays is well recognised. In the Federal Republic of Germany, both external and internal quality-control measures are laid down statutorily for clinical chemistry (1). EQCS for clinical chemical parameters and for several hormones are carried out by two authorised bodies, namely the Deutsche Gesellschaft für Klinische Chemie (German Society for Clinical Chemistry) and INSTAND, Institut für Standardisierung und Dokumentation. The hormone EQCS carried out by these two societies are not obligatory, thus leaving laboratories free to choose whether they participate or not. The hormone EQCS are carried out at regular intervals (3–4 times per year) and consist of two lyophilised samples sent by normal post to participants. An analysis of the results is then sent to each participating laboratory. Both the above organisers charge a small fee to participants to help cover costs involved.

The disadvantage of such EQCS is the limited information which can be given regarding possible sources of error in the assay system of a participant and for this reason, an EQCS model was devised in this laboratory (2–4) to support the above surveys, while at the same time monitoring performance of commercial kits and self-constructed assays. This system has been copied by others (5) and has been given the name the "Munich-Model" of EQCS (5).

Preparation for the EQCS

Choice of participants

Letters of invitation were sent to active members of the Deutsche Gesellschaft für Endokrinologie and the Deutsche Gesellschaft für Klinische Chemie, to all commercial kit producers as well as to selected private and hospital laboratories. Each potential participant was asked whether he wished to take part in a quality control survey for cortisol, and also to indicate his preference for the hormones which he would like to measure in the subsequent 4 EQCS. From the replies, a list of participants as well as a priority list for the next EQCS was drawn up. All information on participants in this and subsequent EQCS was then transferred to punched cards and stored away until needed.

Sample preparation

Serum samples from "special-interest" patients were pooled over a three month period to provide enough material for the EQCS. In addition, possible interfering substances, as well as sera from suppression and stimulation tests were collected. Table 1 shows the composition of the 20 sera sent to each participant. The numbering of the samples was random.

All samples were filtered through an asbestos filter (Seitz-filter, Bender and Hobein, D-8000 München) under pressure (5 Bar N_2) before being portioned into plastic cups (Eppendorf, D-2000 Hamburg) in 1 ml lots. The filtration removed both bacteria and fibrin clots and rendered the sera clear and easy to pipette. All sera were then frozen at $-25^\circ C$ until dispatch.

The medium for the standard curve was made up as follows:

- 40 g $\cdot l^{-1}$ Human Serum Albumin (Sigma – D-8011 Taufkirchen)
- 20 g $\cdot l^{-1}$ Human γ -globulins (Miles-Pentax – D-6000 Frankfurt a.M.)
- 10 g $\cdot l^{-1}$ Human β -globulins (Miles-Pentax)

Tab. 1. Composition of the 20 serum samples dispatched in the cortisol EQCS.

Serum no.	Contents
1	Dexamethasone-suppression test pool serum
2	Hidden standard curve – 0 nmol $\cdot l^{-1}$
3	Pregnancy pool serum – 21–42 weeks of gestation
4	Intra-assay C.V. pool serum
5	Hidden standard curve – 138 nmol $\cdot l^{-1}$
6	Hidden standard curve – 1725 nmol $\cdot l^{-1}$
7	Hidden standard curve – 448 nmol $\cdot l^{-1}$
8	Hidden standard curve – 276 nmol $\cdot l^{-1}$
9	Hidden standard curve – 46 nmol $\cdot l^{-1}$
10	Cushing's disease pool serum
11	Hidden standard curve – 690 nmol $\cdot l^{-1}$
12	Serum 1 + corticosterone – 700 nmol $\cdot l^{-1}$
13	Stripped serum
14	Intra-assay C.V. pool serum
15	Serum 13 + serum 19 1 + 1 mixture
16	Pool serum from 100 patients
17	Intra-assay C.V. pool serum
18	Serum 1 + 11-deoxycorticosterone – 725 nmol $\cdot l^{-1}$
19	Corticotropin stimulation test pool serum
20	Pool serum – upper limit of normal range (09 ⁰⁰ value)

dissolved in *Ringer* solution (Fresenius – D-6380 Bad Homburg) This system gave a zero reading in three different assay systems, namely radioimmunoassay, competitive protein binding and fluorimetry. Corticosterone and 11-deoxycorticosterone were obtained from Fluka, D-7910 Neu-Ulm.

Dispatch

Samples were packed in expanded polystyrene squares and sealed with adhesive foil before being transferred to boxes of the same material (Febra, D-7129 Brackenheim) containing dry ice (5–7 kg). The boxes were then sent by express post or airmail to each participant together with a questionnaire and letter giving the last date for return of completed data forms.

Questionnaire

The points covered by the questionnaire were as follows:

1. Name and address of participant.
2. Whether the sera arrived still frozen.
3. Method used in assaying the samples – including make of kit, label used, extraction procedure.
4. Incubation scheme and volumes and constitution of reagents.
5. Separation of bound and free hormone
6. Counting apparatus and procedure
7. Construction of standard curve with mathematical transformation of data.
8. Data concerning normal range, internal quality-control measures.
9. Invitation to criticise the format of the questionnaire.
10. Results from laboratory-own standard curve in terms of concentrations and counts.
11. Cortisol contractions measured in the 20 sera together with counts.

Data Processing

Treatment of completed questionnaires

All answers and data were coded and transferred to punched cards. The assay details were contained on three cards and the raw data from laboratory standard curve and measured sera on a further 27–32 cards, depending on the number of points used in the laboratory standard curve.

Each participant was allotted a number known only to him and to the organisers. Numbers were allotted in the order in which the

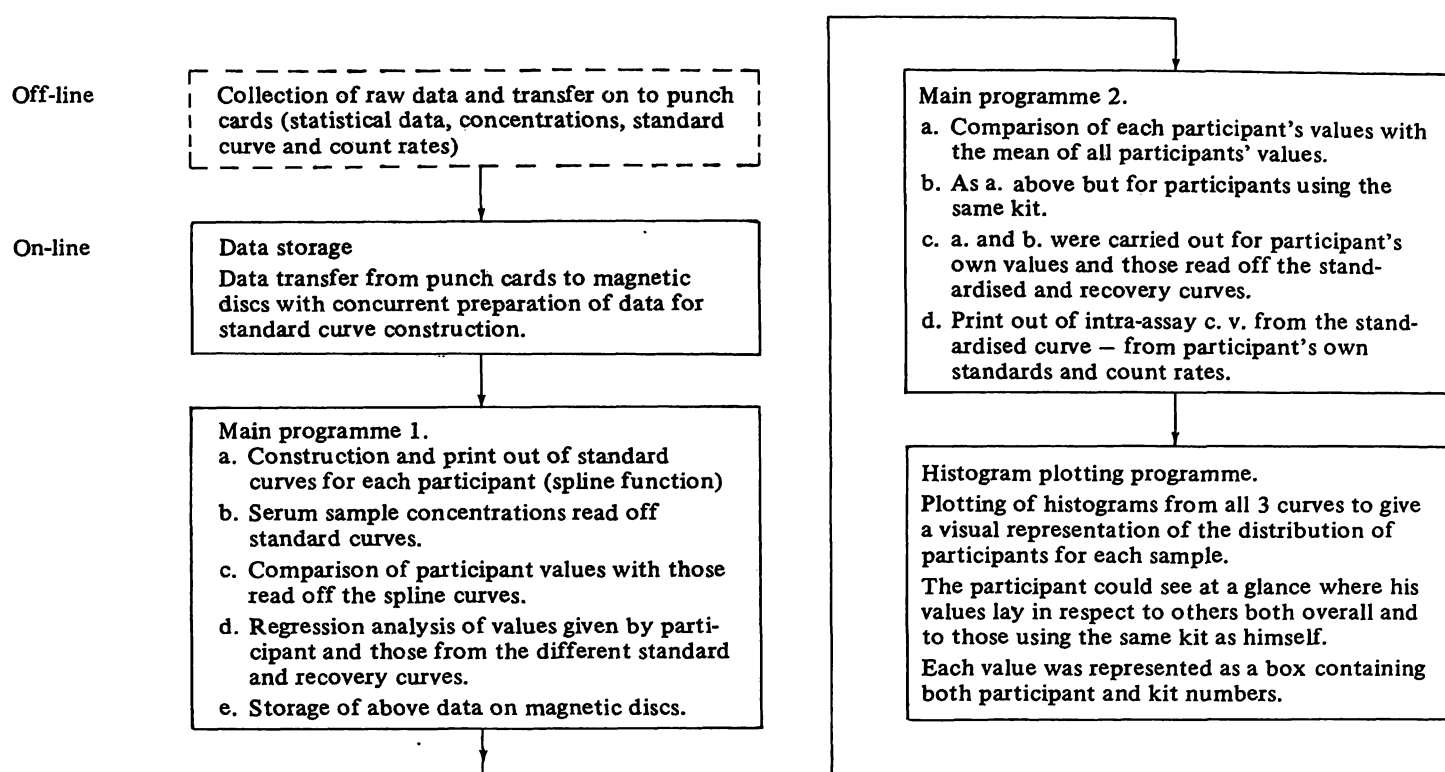


Fig. 1. Data flow sheet and computer programme blocks for EQCS carried out in this laboratory.

returned data were received. The assay method (or kit) was coded to allow a later comparison of performance between kits, and to compare different methods, for example, radioimmunoassay and competitive protein binding assays.

Computer programme layout

Figure 1 shows the layout of the computer programmes. The system was so devised that it could be used for subsequent EQCS with minimal modification. A Siemens 404/3 computer with paper-tape and punched-card reader was used. Data were stored on magnetic discs, each having a capacity of 3 MByte and the programme language was Fortran IV.

The standard and recovery curves were constructed using a spline function as described by *Reinsch* (6) and modified for radioimmunoassay data by *Marschner et al.* (7). Histogrammes were drawn using a plotter connected on-line to the computer.

Treatment of results

After plotting the results and evaluation of data, the results from each participant were examined separately to see if either systematic or random errors were present. Figures 2a–2f show a copy of the 6-page computer print out. An explanatory letter was sent to each participant, containing an evaluation of laboratory performance together with constructive criticism for possible improvement where necessary, and a copy of histograms for all 20 samples. The participant was invited, if he felt it necessary, to contact the organisers if any point was not clear, or if he needed help in reconstruction of his assay in order to obtain better results.

Results

Preliminary Data

150 letters were sent to various laboratories in the Federal Republic of Germany who had indicated interest in taking part in hormone EQCS. 87 laboratories wished

to take part in a cortisol EQCS, of whom 64 returned either fully or partially completed questionnaires. From these 64 participants 49 were able to return fully-useable data and 15 partly-useable data. An analysis of laboratory type and returned data is shown in table 2. The majority of returned data was from University laboratories (50.0 %) with routine and research functions. This group also returned the highest percentage (37.5 %) of incorrectly answered questionnaires.

Tab. 2. Analysis of participating laboratories showing data returned. Laboratories are subdivided into groups.

Type of laboratory	No. of participants	Data fully useable	Data partly useable
University clinic routine laboratories	32	20	12
Local hospital routine laboratories	8	7	1
Large private laboratories	8	6	2
Small private laboratories	2	2	0
Commercial kit producers or representatives	6	6	0
Research laboratories	8	8	0
Totals	64	49	15

TEILNEHMER NR. 1

AUSWERTUNG DES CORTISOL-RINGVERSUCHS MAERZ-APRIL 1977

ALLE KURVEN WURDEN MIT SPLINE-APPROXIMATION ERRECHNET.

KURVE I : STANDARDKURVE NACH ANGABEN DES TEILNEHMERS,
(FEHLTE ANGABE ZUR UNSPEZ.BINDUNG, SO WURDE AUS PROGRAMMTECHNISCHEN GRUENDEN $n = 10$ GESETZT)
(EBENSO WURDE FUER FEHLENDE B_0 -WENDE EIN PROGRAMMTECHNISCH BRAUCHBARER WERT ANGENOMMEN)

KURVE II : VERSTECKTE STANDARDKURVE : NR.2 = 80 , STANDARDS : NR. 9 5 8 7 11 6
MIT DEN VOM VERANSTALTER EINGESETZTEN KONZENTRATIONEN: 46 138 276 448 690 1725 (NMOL/L)
UND DEN VOM TEILNEHMER GEMESSENEN ZAENLRATEN.

KURVE III : VERSTECKTE STANDARDKURVE AUS DEN GLEICHEN PROBEN, ABER MIT DEN VOM TEILNEHMER GEMESSENEN
KONZENTRATIONEN, (PROZ.BINDUNG BEZUGEN AUF DAS B_0 DER STANDARDKURVE)

KONZENTRATIONEN, DIE FUER DIE KURVEN II ODER III EINE NICHT ANSTIEGENDE KONZENTRATIONSFOLGE ERGEBEN HÄTTEN,
WURDEN NICHT BERUECKSICHTIGT.

KURVE I

STANDARDKONZENTR.	PROZ.(B ₀)	S.D.	2*S.E.M.	ORDINATE	ZAENLRATEN				
STANDARDLEERWERT(B ₀)	100.00	2.496	2.255	100.63	8675.	8740.	9072.	9136.	6762.
27.6 NMOL/L	92.43	2.872	2.872	89.30	8105.	8105.	8142.	8601.	
82.8 NMOL/L	68.85	2.108	2.108	68.47	6365.	6189.	6413.	6023.	
165.6 NMOL/L	53.46	0.537	0.537	53.60	4979.	4903.	4920.	4997.	
276.0 NMOL/L	44.35	3.066	3.066	43.82	4248.	4453.	4183.	3831.	
690.0 NMOL/L	28.79	1.656	1.656	29.11	2993.	2973.	2878.	2687.	
1656.0 NMOL/L	17.95	1.488	1.488	17.90	2058.	1769.	1996.	1978.	
3312.0 NMOL/L	10.27	1.381	1.381	10.30	1267.	1296.	1184.	1462.	
UNSPEZ.BINDUNG(N)					438.	316.	480.	507.	

KURVE II

STANDARDKONZENTR.	PROZ.(B ₀)	S.D.	2*S.E.M.	ORDINATE	ZAENLRATEN		
STANDARDLEERWERT(B ₀)	100.00	1.369	1.581	100.01	9131.	9325.	9106.
46.0 NMOL/L	75.61	0.899	1.039	73.79	6977.	6820.	6888.
138.0 NMOL/L	54.52	1.093	1.262	54.36	5317.	5148.	5155.
276.0 NMOL/L	40.57	0.807	0.932	40.90	4067.	3937.	3954.
448.0 NMOL/L	34.63	1.297	1.497	33.23	3585.	3359.	3454.
690.0 NMOL/L	24.78	0.822	0.949	25.18	2566.	2687.	2559.
1725.0 NMOL/L	14.12	0.919	1.061	14.01	1759.	1654.	1601.

KURVE III

STANDARDKONZENTR.	PROZ.(B ₀)	S.D.	2*S.E.M.	ORDINATE	ZAENLRATEN		
STANDARDLEERWERT(B ₀)	103.68	1.420	1.659	103.97	9131.	9325.	9106.
56.9 NMOL/L	76.52	0.933	1.077	75.57	6977.	6820.	6888.
144.0 NMOL/L	56.52	1.133	1.308	57.13	5317.	5148.	5155.
305.0 NMOL/L	42.06	0.837	0.966	42.43	4067.	3937.	3954.
441.0 NMOL/L	35.90	1.344	1.552	36.07	3585.	3359.	3454.
885.0 NMOL/L	25.69	0.852	0.984	25.73	2566.	2687.	2559.
2215.0 NMOL/L	14.64	0.953	1.100	14.59	1759.	1654.	1601.

Fig. 2a.

Fig. 2a-2f. A typical computer-print-out.

Fig. 2a. The explanation of the 3 curves used, and the data used in constructing the curves. Curve I is from the data and counts of the participant's own standard curve; curve II is the hidden standard curve using the organisers' assigned values and count-rates from the participant; curve III the values of the hidden standards measured by the participant and his own count-rates. Here B_0 is calculated as a percentage of the count-rates of the zero standard in curve I.

Methods and kit codes

43 participants used commercially available kits and 21 their own methods. 48 participants used radioimmunoassay (RIA), 15 used competitive protein binding analysis (CPBA) and one fluorimetry to measure cortisol. 36 participants used ^{125}I , 20 used ^3H and 8 used ^{75}Se labelled tracer. The code used for the kits was:

1. Clinical Assays (RIA — ^{125}I -tracer) Travenol, D-8000 München.
2. Diagnostic Products Corporation (RIA — ^{125}I -tracer) Biosigma, D-8000 München

3. Amersham-Buchler (CPBA — ^{75}Se -tracer) D-3300 Braunschweig.
4. Coming (RIA — ^{125}I -tracer) IMA, D-6300 Gießen
5. Byk-Mallinckrodt (RIA — ^{125}I -tracer) D-6057 Dietzenbach-Steinberg.
6. Clinical Assays — Modified method — otherwise as Kit 1.
7. CIS (CPBA — ^{125}I -tracer) Isotopen Dienst West, D-6079 Sprendlingen.
8. Lepetit (CPBA — ^3H -tracer) Milan, Italy.
9. New England Nuclear (RIA — ^{125}I -tracer) D-6072 Dreieich.

Own methods were not coded.

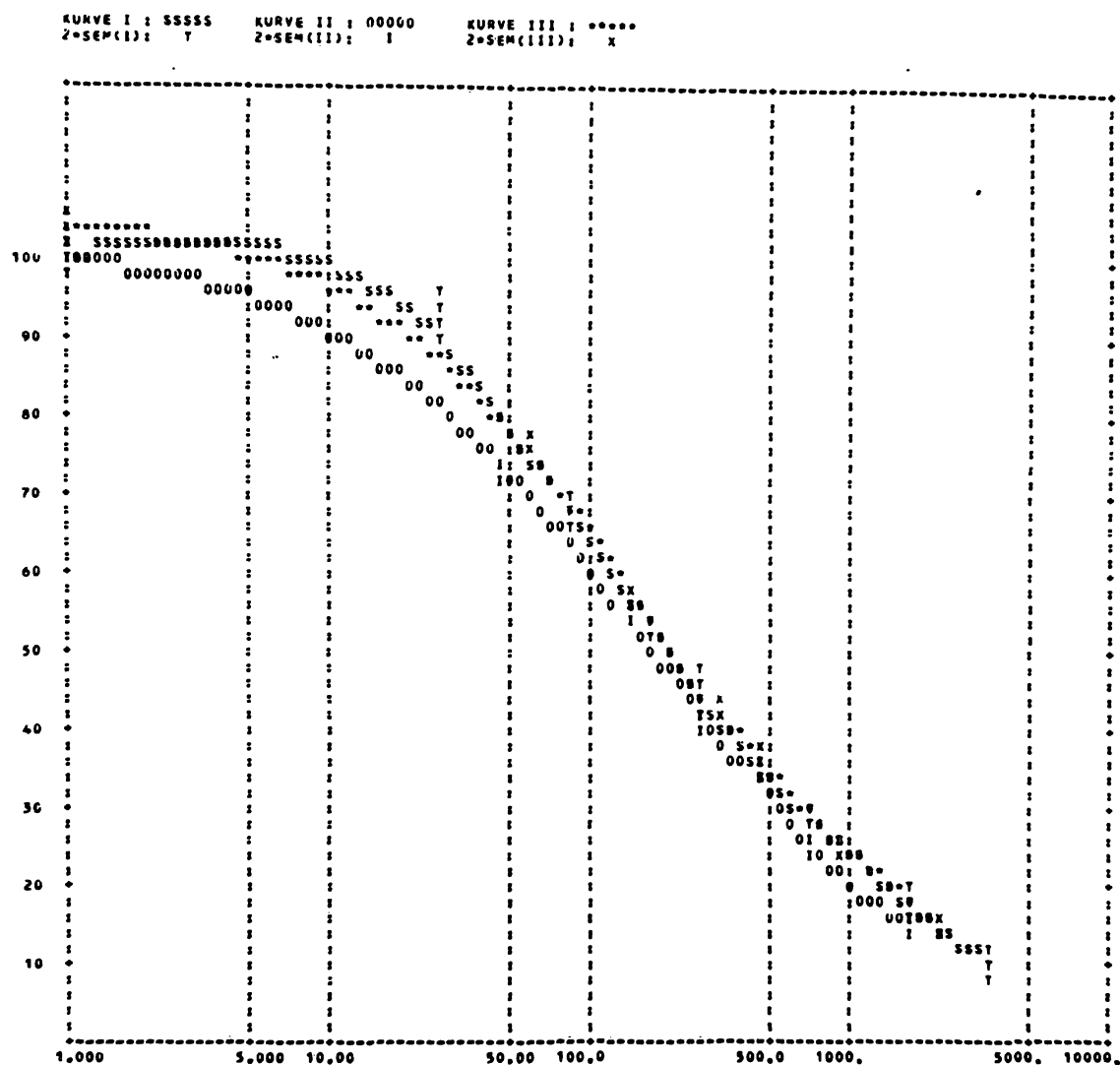


Fig. 2b. The print-out of the 3 curves.

Results of sample analysis

The content of the 20 serum samples is shown in table 1. The results of the more interesting samples are displayed in table 3. Difference in results from samples 2 and 13 are due to the different matrix in the so-called cortisol-free serum. Table 3 shows the results of samples either used for testing dilution or cross-reacting substances added to a serum sample.

Samples 1, 12 and 18 should be approximately the same if no cross-reactivity occurred between the antibody and corticosterone and 11-deoxycorticosterone, in the RIA, or if the binding proteins were specific for cortisol in the CPBA. In all CPBA the cross-reaction in samples 12 and 18 was high, reflecting the relatively unspecific nature of cortisol-binding-globulin (CBG) for these compounds.

The kit CPBA gave on average lower cross reactivity than the "home-produced" CPBA. The one participant who

used fluorimetry to measure cortisol measured corticosterone, but not 11-deoxycorticosterone, showing that his extraction method was specific for 11-hydroxy steroids.

The cross-reaction of the RIA-methods in samples 12 and 18 was varied, and depended to some extent on the derivative used to raise the antiserum. The antibodies raised to C₃-derivatives were more specific than those raised to C₂₁-derivatives. The kits showing no cross-reactivity in both samples 12 and 18 (kits 2 and 6) were raised against C₃-derivatives.

The cortisol measured in samples 2 and 13 was similar, although on average sample 13 (stripped serum) gave a lower value than sample 2 (protein matrix). Both sample 2 and 13 gave significantly lower values than sample 1 (dexamethasone suppression-test).

In all cases where no interfering substances were present, all methods gave similar results seen in samples 13, 15

TEILNEHMER NR. 1

AUSWERTUNG DES CORTISOL-RINGVERSUCHS MAERZ-APRIL 77 TEIL 2

IN DER NACHFOLGENDEN LISTE IST DEN UNGEFAEHRN CORTISOL-GEHALT BZW. DIE HERSTELLUNG DER PROBEN AUFGEFUEHRT, DIE MIT 'STANDARD' BEZEICHNETEN PROBEN DIENEN ZUR GEWINNUNG DER 'VERSTECKTEN' STANDARDKURVEN, (SIEHE TEIL 1) DIE MIT 'TEST' ODER 'POOL' BEZEICHNETEN PROBEN DIENEN ZUR KONTROLLE DES ASSAY-VERHALTENS UNTER VERSCHIEDENEN BEDINGUNGEN, DIE SICH STOEREND AUSWIRKEN KONNTEN, IM EINZELNEN HABEN DIE PROBEN FOLGENDE BEDEUTUNG :

PROBE 2 UND 13 SOLLTEN BEIDE ALS 'CORTISOLFREI' ERMITTELT WERDEN,

DIE PROBEN 4, 14 UND 17 WAREN GLEICH (IM MITTLEREN BEREICH) UND DIENEN ZUR INTRA-ASSAY-QUALITAETSKONTROLLE,

DIE PROBEN 12 UND 18 DIENEN ZUR PRUEFUNG AUF EVTL. KREUZREAKTIONEN, SIE HATTEN DEN GLEICHEN CORTISOL-GEHALT WIE PROBE 1, WAREN ABER MIT STOERSUBSTANZEN VERMISCHT,

PROBE 15 SOLLTE ETWA DEN MITTELWERT ZWISCHEN PROBE 13 UND 19 ERGEBEN,

DIE PROBE 16 DIENTE ZUR PRUEFUNG UNSPEZIFISCHER STOEREFFEKTE,

1 TEST DEXAMETHASON SUPPRIMIERT	
2 STANDARD	0 NMOL/L
3 TEST SCHWANGENSCHAFT 2. HAELFTE	
4 POOL NORMALBEREICH	
5 STANDARD	138 NMOL/L
6 STANDARD	1725 NMOL/L
7 STANDARD	448 NMOL/L
8 STANDARD	276 NMOL/L
9 STANDARD	46 NMOL/L
10 TEST CUSHING-SYNDROM	
11 STANDARD	690 NMOL/L
12 TEST DEXAMETHASON + CORTICOSTERON	700 NMOL/L
13 TEST 'STRIPPED SERUM' (0-WERT)	
14 POOL NORMALBEREICH	
15 TEST ACTH + 'STRIPPED SERUM' 1:1	
16 TEST MISCHSERUM VON 100 PATIENTEN	
17 POOL NORMALBEREICH	
18 TEST DEXAMETHASON + 11-DESOXYCORTICOSTERON	725 NMOL/L
19 TEST ACTH-STIMULIERT	
20 POOL GRENZWERT/ERNUEWT	

Fig. 2d. The composition of the 20 sera.

and 19, where 15 lay, as expected midway between 13 and 19.

In contrast to an earlier EQCS for thyrotropin (2) the results from the hidden standard curve were not much better than those from the standardised laboratory-own curve. This is perhaps due to the fact that the antibodies used were raised against different cortisol-protein conjugates, that all methods involved a prior extraction/denaturation step and the relative unspecific binding characteristics of CBG. The use of a non-ideal matrix in which the standard curve in the survey samples was set up had most probably little or no influence on the results when the assay conditions are examined.

The results for sample 17 are shown in figures 3a-3c as constructed from laboratory results, from the standardised laboratory result and from the hidden standard curve. These pictures emphasise what is written in the previous paragraph.

Coincidence of standardised laboratory curve and hidden standard curve occurred in 28 cases (56 %). The

regression equation as read off the laboratory own and standardised laboratory curve was better than $y = a + (0.9 \text{ to } 1.1) x$ in 36 cases (72 %), an index for the ability to read data from a standard curve. Within the same limits, the regression line between standardised laboratory curve and hidden recovery curve was identical in only 13 cases (26 %), and serves as an index for the accuracy of the assay.

The number of participants with an intra-assay coefficient of variation (CV) under 5% was 21 (33 %), between 5 and 10 %, 16 (25 %), between 10 and 15 %, 7 (11 %) and above 15 %, 20 (31 %). The minimum CV recorded was 0 % and the maximum 86 %.

Discussion

The amount of data returned in the "Munich-Model" EQCS is enormous and it is difficult to extract only those data which give the maximum information to the participant. The visual aid effect of the standard and hidden standard curves show the participant at a glance

TEILNEHMER NR. 1 EIGENE ANGABEN :									

NR.	INR WERT :	ALLE TEILNEHMER			TEILNEHMER MIT IHREM KIT (NR 1)				
		MITTELWERT	S.D.	2*S.D.-BEREICH	MITTELWERT	S.D.	2*S.D.-BEREICH		
1	29.50	37.20	15.98	5.24 - 69.16	33.18	10.08	13.01 - 55.35		
2	0.00	25.53	22.00	-18.04 - 69.94	15.58	14.33	-13.07 - 44.23		
3	1004.00	919.76	173.21	573.35 - 1266.17	1034.23	107.98	818.27 - 1250.19		
4	243.00	236.11	47.36	141.38 - 330.84	241.84	37.29	167.26 - 316.42		
5	144.00	172.35	49.57	73.21 - 271.49	168.89	27.40	114.09 - 223.69		
6	2215.00	1726.75	363.98	998.80 - 2454.70	2158.57	134.75	1889.07 - 2428.07		
7	441.00	500.89	121.08	258.73 - 743.05	511.01	86.64	337.73 - 684.29		
8	305.00	342.37	112.72	116.93 - 567.81	330.59	55.46	219.68 - 441.50		
9	56.90	62.31	32.75	-3.18 - 127.80	58.51	25.21	8.10 - 108.92		
10	1137.00	878.24	148.54	581.16 - 1175.32	987.07	121.46	744.14 - 1230.00		
11	865.00	783.95	206.12	371.71 - 1196.19	827.17	110.16	606.86 - 1047.48		
12	114.00	176.44	161.38	-146.32 - 499.20	93.66	18.38	56.91 - 130.41		
13	12.10	22.07	16.11	-10.16 - 54.30	10.38	4.04	2.29 - 18.47		
14	262.00	234.31	54.33	125.65 - 342.97	232.04	51.33	129.38 - 334.70		
15	401.00	368.93	84.37	200.20 - 537.66	343.71	37.15	269.42 - 418.00		
16	1569.00	773.47	507.99	157.50 - 1349.44	1216.08	194.23	827.62 - 1604.54		
17	244.00	236.14	61.55	113.05 - 359.23	242.52	62.04	118.43 - 366.61		
18	273.00	294.13	204.02	-113.91 - 702.17	364.43	58.69	247.05 - 481.81		
19	891.00	722.47	154.15	414.16 - 1030.78	763.78	179.93	403.91 - 1123.65		
20	662.00	548.28	134.08	280.12 - 816.44	585.09	60.29	464.51 - 705.67		

INTRA-ASSAY-VARIANZ AUS DEN PROBEN : 4, 14, 17,

MITTELWERT= 249.67 S.D.= 10.693 V.K.= 4.28 PROZ.

ABGELESEN VON KURVE I :

NR.	INR WERT :	ALLE TEILNEHMER			TEILNEHMER MIT IHREM KIT (NR 1)				
		MITTELWERT	S.D.	2*S.D.-BEREICH	MITTELWERT	S.D.	2*S.D.-BEREICH		
1	29.52	32.80	18.31	-3.81 - 69.41	25.43	9.84	5.77 - 45.13		
2	0.00	23.50	22.98	-22.45 - 69.45	11.37	9.66	-7.96 - 30.70		
3	1003.05	940.29	160.92	618.45 - 1262.13	1069.73	109.83	850.02 - 1289.44		
4	243.01	227.61	42.09	143.42 - 311.80	226.65	34.46	157.74 - 295.56		
5	143.87	155.86	44.69	66.49 - 245.23	147.20	41.42	64.35 - 230.05		
6	2209.16	1860.82	338.71	1183.41 - 2538.23	2132.19	208.78	1714.64 - 2549.74		
7	440.30	485.33	108.71	267.92 - 702.74	507.11	88.52	330.07 - 684.15		
8	304.84	307.35	89.76	127.82 - 486.88	320.10	53.37	209.37 - 430.83		
9	56.49	52.28	27.08	-1.89 - 106.45	51.55	28.71	-5.87 - 108.97		
10	1135.67	919.94	137.18	645.59 - 1194.29	999.19	118.08	763.02 - 1235.36		
11	884.64	770.86	167.50	435.86 - 1105.86	860.79	81.13	698.52 - 1023.06		
12	113.47	151.88	129.14	-106.40 - 410.16	85.17	21.90	41.37 - 128.97		
13	10.47	17.04	15.73	-14.45 - 48.51	6.39	4.29	-2.20 - 14.98		
14	211.30	224.87	58.50	107.87 - 341.87	216.94	53.37	108.21 - 321.67		
15	401.81	355.56	94.30	166.97 - 544.15	351.39	74.69	202.02 - 500.76		
16	1386.85	746.85	292.88	161.07 - 1332.59	1264.31	172.46	919.40 - 1609.22		
17	242.90	251.44	73.76	83.90 - 378.94	209.56	33.61	142.34 - 276.78		
18	272.41	295.58	209.29	-123.00 - 714.16	355.30	63.29	228.73 - 481.87		
19	887.00	736.15	166.94	398.24 - 1074.02	784.36	169.67	445.02 - 1123.70		
20	660.43	546.02	119.22	309.59 - 782.45	612.46	97.41	417.63 - 807.29		

Fig. 2e.

TEILNEHMER NR. 1
ABGELESEN VON KURVE II :

NR.	INR WERT :	ALLE TEILNEHMER			TEILNEHMER MIT IHREM KIT (NR 1)				
		MITTELWERT	S.D.	2*S.D.-BEREICH	MITTELWERT	S.D.	2*S.D.-BEREICH		
1	18.52	27.10	15.93	-4.76 - 58.96	19.38	12.18	-4.98 - 43.74		
2	1.01	10.51	10.95	-11.39 - 32.41	3.80	3.69	-3.59 - 11.19		
3	779.78	837.16	186.89	463.37 - 1210.95	843.54	89.51	664.52 - 1022.56		
4	225.61	187.02	41.80	103.43 - 270.61	198.49	36.42	125.64 - 271.34		
5	136.90	135.73	7.95	119.83 - 151.63	135.15	12.40	110.36 - 159.94		
6	1705.03	1704.38	28.64	1647.10 - 1761.66	1712.21	8.03	1696.14 - 1728.28		
7	412.41	444.02	19.03	405.97 - 482.07	426.12	28.77	368.59 - 485.65		
8	281.61	270.66	23.59	223.48 - 317.84	272.60	16.64	239.32 - 305.88		
9	45.96	41.39	5.56	30.26 - 52.52	46.72	13.16	20.40 - 73.04		
10	866.18	830.27	127.61	575.05 - 1085.49	797.63	53.54	690.54 - 904.72		
11	706.27	702.88	33.42	636.04 - 769.72	695.30	38.37	618.56 - 772.04		
12	106.56	152.14	173.82	-195.50 - 499.78	78.28	35.97	6.35 - 150.21		
13	4.49	16.18	9.08	-1.98 - 34.34	2.89	2.26	-1.63 - 7.41		
14	197.95	183.93	36.75	110.44 - 257.42	190.22	40.74	108.74 - 271.70		
15	376.84	324.86	77.12	170.63 - 479.09	303.73	62.34	179.05 - 428.41		
16	1043.09	723.64	270.37	182.89 - 1264.59	979.68	106.10	767.49 - 1191.87		
17	225.52	193.40	45.25	102.90 - 283.90	202.64	46.08	110.47 - 294.81		
18	251.66	256.22	180.31	-104.40 - 616.84	303.80	28.70	246.40 - 361.20		
19	707.71	672.44	124.01	423.21 - 921.67	638.04	135.90	366.25 - 909.83		
20	570.90	495.35	81.64	332.07 - 658.63	485.68	51.50	382.69 - 588.67		

ZEICHENERKLÄRUNG :

"•" : WERT LIEGT AUSSERHALB DES 2*S.D.-BEREICHS ALLER TEILNEHMER.

"••" : WERT WAK NICHT MESSBAR.

Fig. 2f.

Fig. 2e-2f. Values determined by the participant compared with values obtained by other participants using the same kit, and read off the participant's standard curve, the standardised (curve I) and the recovery (curve II) curves. The mean, standard deviation (S) and $\pm 2S$ ranges are given.

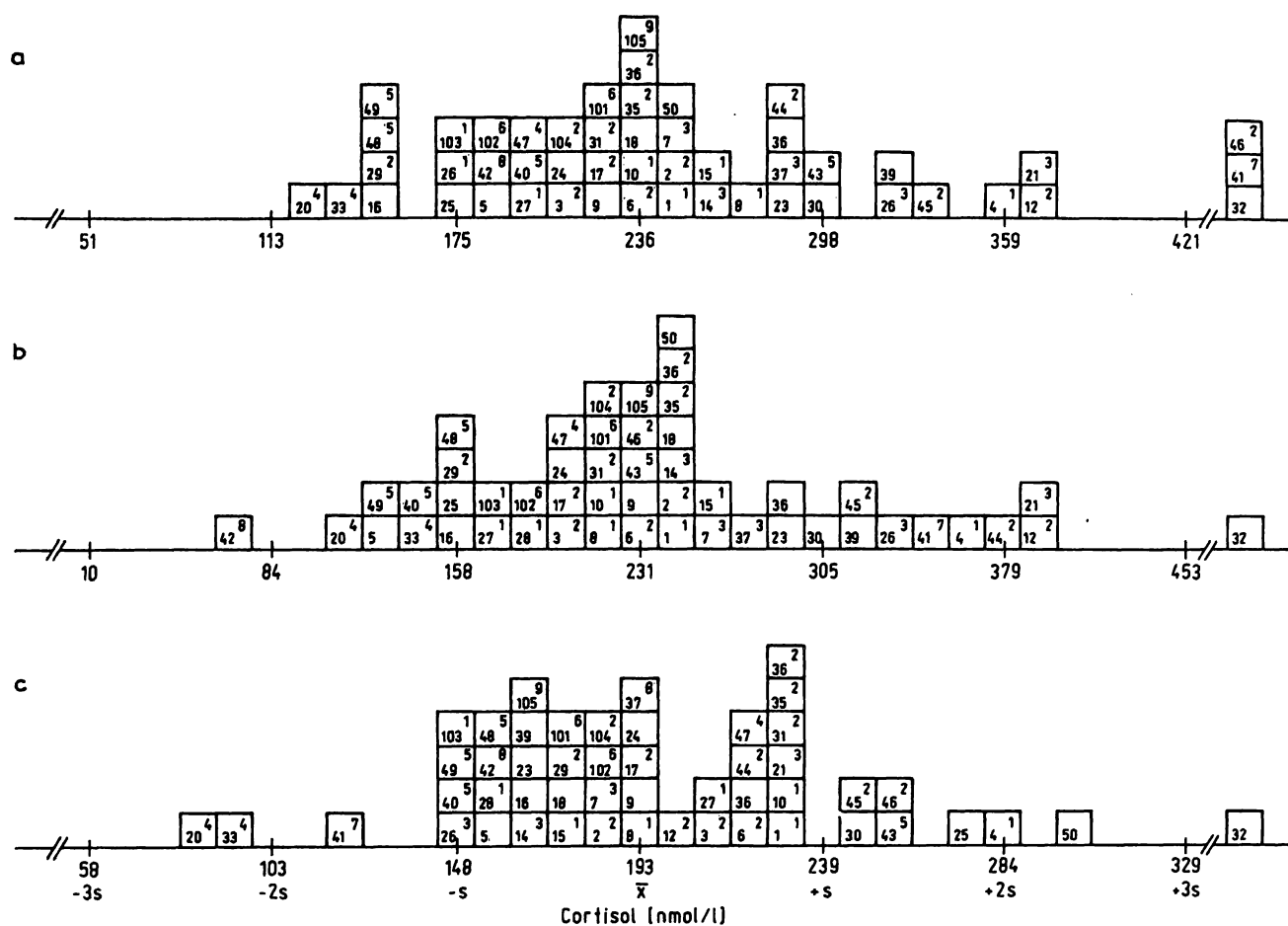


Fig. 3a–3c. The distribution of sample 17 (pool serum) from results given by the participant (fig. 3a), read off the standardised curve (fig. 3b) and from the hidden recovery curve (fig. 3c). The abscissa values are mean \pm 3S range. The figures in the boxes are: lower left, participant number and upper right, kit number. Values lying outside the \pm 3S range are shown on the extreme left and right of the histograms.

if his assay is in order or not, although it must be stated that this is only strictly true for this one assay. This is in itself a disadvantage of the "Munich-Model" EQCS, which can only perform a spot-check on how a laboratory performs. This information is very important, especially in a country in which a large number of commercial kits are used. Changes in protocol, antibody, and tracer are not infrequent, and these can give rise to different results as seen in the comparison between kits 1 and 6 in this survey where new antibody, tracer and protocol considerably reduced the cross reactivity between 11-deoxycorticosterone and cortisol.

Another disadvantage of this system is its time-consumption and its limitation to one, or at the most two components. This means that the same component cannot be repeated inside a 2-year period unless it is carried out by an institute specially designed to perform such quality control surveys. A few examples will make this more clear. The data from each participant is transferred to between 30 and 35 punched cards (20 for the samples, 3 for the assay data and

between 7 and 12 for the laboratory-own standard curve, including total counts and unspecific binding). The modification of the computer programmes has been minimised by the modular construction used, but around 100 punched cards are needed for the headings and serum composition, together with 20 cards for each kit, containing mean and s.d. for each sample, and 60 cards containing sample number mean and s.d. for the histograms. The first 100 text-cards must be written by hand, the latter 80 data cards are punched by commands from the computer. Each participant's printout of 6 pages took approximately 3½ minutes, most of this time being spent on the curve plotting, and each histogramme – in this EQCS – took between 10 and 11 minutes. Together with the photo-copying time needed so that each participant received the 20 histograms for the samples and an example read from the standardised and hidden standard curve, one can begin to appreciate the real time needed – simply for sending the results back to each participant.

As can be seen, this type of survey must be coupled to a regular 2 or 3 sample EQCS, which are at present run in

many countries, together with a continuous and rigorous laboratory internal quality control scheme.

Another interesting point in the "Munich-Model" is the provision of correlation data between own results and those from the standardised and hidden standard curves. Here, it is very easy to see if errors which have arisen are of a methodological or random nature. For example a good correlation coefficient with a regression line slope widely deviating from 1.0 points to a methodological error (perhaps wrong dilution of standards) whereas a bad correlation coefficient indicates a non-optimal assay system.

This type of EQCS has had a positive spin-off effect, that of monitoring the quality of commercial kits, without having to do anything except send the results to each participant. The effects of this, and of earlier EQCS from this laboratory (2) have led to a subsequent improvement of methodology and quality in commercial kits, and this method of "automatic-policing" is far more effective and much cheaper and quicker than legislative measures, which must be constantly updated to keep in touch with a rapidly moving field such as hormone analysis.

The two-way contact opened up between kit-producers and organisers in this type of EQCS has led to a positive dialogue often resulting in improvement in kit-performance by optimisation of methodology, thus paving the way for comparable results between different kits, a goal which must be attempted, especially for peptide and proteohormones. That this state of affairs is by no means achieved even for a small molecule like cortisol, can be

seen by the wide variation in individual results and the relatively low coincidence of hidden and laboratory-own standard curves (26 %). The use of non-specific methods, here the CPB assays, show in their high cross-reactivity with closely related compounds, that many laboratories still use techniques which have long since been superseded by more modern technology – in this case RIA and ^{125}I -labelling, which give remarkably good results and are to be recommended, at least until the next generation of tests has been developed and proofed.

The participant, for relatively little cost and work-load, receives a large amount of data, with which he can examine his method for faults, if such have arisen, and with which he can compare his performance with other methods and participants. The complete anonymity of participants, reduces the inhibition to make contact with the organisers should a participant require help, which is a very important point. To conclude, although these EQCS are laborious, they provide enough positive influence on the current state of hormone assays to warrant their continuance.

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